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Twin-screw Extrusion Processing of Rainbow trout (*Oncorhynchus mykiss*) feeds using Graded Levels of High Protein Corn-based Distillers Dried Grains (HP-DDG) and Conventional Distillers Dried Grains with Solubles (DDGS)

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Abstract. *Twin-screw extrusion cooking trials were performed to investigate the differential effects of conventional DDGS (DDGS) and HP-DDG inclusion, as fishmeal replacers, on physical properties (moisture content, water activity, thermal properties, expansion ratio, unit density, bulk density, water absorption, solubility and pellet durability indices, and color) of Rainbow trout (*Oncorhynchus mykiss*) feed. Four ingredient blends were formulated with 20 and 40% C- DDGS, and 20 and 40% HP-DDG, along with other required dietary ingredients. Each diet was extruded using two replications; a fishmeal based diet acted as the control diet. The highest and the lowest extrudate moisture content and water activity were observed for the diets containing 20% DDGS and 40% HP-DDG, respectively. Compared to the control diet, increasing HP-DDG content from 20 to 40% had no effect on the bulk density of the products, while increasing DDGS content from 20-40% led to a considerable rise in bulk density of the extrudates by 14.2 and 6%, respectively. Also, extrudates*

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with the lowest brightness, greenness, and yellowness values were obtained from the diet containing 20% DDGS, whereas the most color intensity values were observed for the diets containing 40% DDGS and 20% HP-DDG. Increasing DDGS and HP-DDG from 0-40% caused a remarkable increase in water absorption index, by 72 and 30%, respectively. Likewise, the water solubility index increased, but in a considerably lesser order of magnitude. High pellet durability index of more than 99% was achieved with inclusion of either DDGS or HP-DDG. None of the diets impacted the thermal properties of the extrudates. Inclusion of 20 and 40% DDGS resulted in the extrudates with the largest and the smallest unit density values of nearly 887 and 750 kg/m³, respectively. As was expected, expansion ratio decreased with increasing unit density. Increasing inclusion levels of HP-DDG did not show any effect on unit density or expansion ratio of the products. Future research should concentrate on investigating the effects of both extrusion processing and graded levels of different types of DDGS on Rainbow trout feeds.

Keywords: *Alternative protein, Aquaculture, Ethanol co-product, Extrudate, High protein, Trout*

1. Introduction

Expanding the aquaculture industry enhances the global food security and subsequently helps boost the living standards of the world's growing population; however, the importance of the techno-economic feasibility of aquaculture production must be considered (Pritchard, 1976). Increasing consumer demands for aquaculture products coupled with a limited food supply (i.e. wild fish supply) for feeding the fish have resulted in a remarkable increase in aquafarm production expenses. The largest part of these costs comes for feed in which protein is the most expensive ingredient (Watanabe et al., 2002). The required protein content of the feed varies depending on the species and size of the cultured fish (Craig and Helfrich, 2002; NRC, 1993), which, in turn, strongly impacts the fish growth performance and thus production efficiency. Fish meal, which is the best source of protein and oil due to its high biological values in terms of essential amino acid, unsaturated fatty acids, minerals, and phospholipids for fish metabolism, comes largely from wild fish stocks, (Cheng and Hardy, 2004a). In general, anywhere from 2 to 6 pounds of ocean fish must be used to produce only one pound of farm-raised fish, resulting in a 25% average transfer efficiency (Marine Aquaculture Task Force, 2007).

Overexploitation of wild fish stocks to feed the fish not only impacts the ocean ecology balance but increases product costs and reduces the profitability of the aquaculture industry. One way to make the aquaculture industry more environmentally sustainable is to lower the amount of fish meal in fish diets or to stop feeding the fish by fish. Hence, further development of the aquaculture industry as one of the key pillars of sustainable world economic growth requires the substitution alternative protein sources for fish meal.

Since 1950, exploring alternative protein sources to enhance the sustainability of the aquaculture sector has become an area of great interest. Research with the goal of partially or even complete elimination of fish meal in aquafeeds has been conducted to substitute fish meal with other potential sources of protein such as oilseeds, meat byproducts and microbial protein sources (Aas et al., 2006; Coyle et al., 2004; Hardy, 2010; Hardy & Masumoto, 1990; Jauncey, 2000; Naylor et al., 2005; Rumsey, 1993; Samocha et al., 2004; Shapawi et al., 2007; Storebakken et al., 2004; Webster et al., 1999). It is consensus among the aquaculture scientists that an increased utilization of vegetable-based proteins would help to overcome the issue as they are abundant and economical (Hardy, 2010). Although inclusion of fish meal replacers can reduce the feed cost, complete elimination of fish meal in the aquafeed, particularly for carnivorous species like Trout, has still been questioned, as it may cause severe problems due to the less palatability, digestibility, and lack of essential amino acid profile of the plant-derived proteins (Lim and Dominy, 1991; Webster et al., 1999). These limiting issues can be influenced by other factors such as the variety of the plant, pretreatment of the plant-based protein before being used in an aquafeed diet formula, and the feed production technology which can affect both biological and physical properties of the feed.

Over the past several years, huge expansion of corn-derived ethanol industry in the U.S. has been accompanied by a remarkable increase in annual production of distillers dried grains (DDGs), a non-fermentable coproduct of ethanol plants. In addition to corn, many other cereal grains such as wheat and barley can be used in ethanol production (Stock et al., 2000; Vander Pol et al., 2005). However, in the Midwest, most ethanol plants use corn as a raw material (Singh et al., 2001). This surge in production of DDGS reinforces the need to achieve a balance between the U.S. supply and

consumption markets (Cooper, 2006). The total production of distillers dried grains with solubles (DDGS) from 2007 to 2011 increased by nearly 65% (AgMRS, 2012). In 2010, the DDGS supply in the US was 33.3 mmt, with a predicted increase to nearly 39 mmt by 2020 (USDA, 2010). DDGS is a valuable source of protein (27-33%) and fiber (5-12%) (Rosentrater and Muthukumarappan, 2006), which has the potential of feeding both the US livestock and economy (Ham et al., 1994; Lim et al., 2009; RFA, 2010; Saunders and Rosentrater, 2009). On the other hand, comparing with the other plant-based protein such as soy products, DDGS has considerably fewer anti-nutritional factors (ANFs) like trypsin inhibitors (TIA) and lectin (Robinson, 1991; Shiau et al., 1987) and better essential amino acid (EAA) profiles; however, it is still suffering from a lack of some EAAs like lysine, methionine, and tryptophan in comparison to those present in fish meal protein (Cheng and Hardy, 2004a; Chhron and Mediha, 2008; Metts et al. 2007). Additionally, lower phytate phosphorous content of DDGS lessens the total unavailable phosphorous level of the feed formula and consequently minimizes water pollution as a result of decreased phosphorous excretion into the water of the pond or aquarium. Typically, animal protein source such as fishmeal or poultry by-products and plant protein source such as soy products contain significant amount of phytate phosphorous which is unavailable to most fish (Davis, 1999). Therefore, phytase supplementation of the diet can lead to easier release of phosphorous from phytate, making it more available to fish and reducing the undigested phosphorous discharge (Cain and Garling, 1995; Cheng and Hardy, 2002; Papatryphon et al., 1999; Rodehutscord and Pfeffer, 1995; Storebakken et al., 1998; Sugiura et al. 2001; Vielma et al., 1998, 2000). In addition, better disease resistance of the fish fed was reported with DDGS for some species like catfish (allaboutfeed.net, 2008; Lim et al., 2007).

In general, two conventional methods exist for ethanol production: wet mill processing and dry mill processing. Due to lower initial investments and recent advancements in fermentation technology, dry milling industries have been growing considerably (Belyea et al., 2004; Spiehs et al., 2002). During the dry-milling process, three types of coproducts including distillers dried grains (DDGs), distillers dried solubles (DDS), and distillers dried grains with solubles (DDGS) are produced after the starch fermentation and distillation of fermented mash. The soluble portion of the residual coproduct is evaporated and condensed distillers solubles (CDS) are produced. The solid portion of the remaining fermentation residue and condensed distillers solubles are both subjected to drying and produce either distillers dried grains (DDG) or distillers dried solubles (DDS) (Singh et al., 2005; University of Minnesota, 2008). When these two coproducts are mixed and dried, distillers dried grains with solubles (DDGS) is produced. DDS is a viable source of vitamin and fat and contains the highest nutrient concentration compared to those of DDGS and DDG (University of Minnesota, 2008). Furthermore, the nutrient amounts in DDGS are almost triple those of raw corn (Jacques et al., 2003) as a result of the fermentation and yeast metabolism. Recently biorefinery plants have employed a new technique to increase the ethanol production yield. In the new dry-milling process, whole corn is fractionated into several fractions including germ, bran, and endosperm. Germ fraction contains high fat and phosphorous concentrations in addition to a better essential amino acids profile. Endosperm fraction is subjected to fermentation and ethanol recovery. Indeed, pre-removal of the nonfermentable fraction of corn is the main reason for the ethanol yield increase (Singh et al., 2005). The coproduct of this process is DDG, which is high in protein but low in fat. Typically, conventional DDGS (DDGS) contains approximately 30% protein (Rosentrater

and Muthukumarappan, 2006; Spiehs et al., 2002). The protein content of DDG is almost 1.5 times that of DDGS; hence, it is called HP-DDG (Robinson et al., 2008). Moreover, using HP-DDG provides higher available phosphorous content thus reducing the need for phosphorous supplementation and subsequently the phosphorous excreted into the water. Robinson et al. (2008) believed that HP-DDG nutritional values are much more consistent than those of DDGS. However, the grain variety and production processing parameters such as over processing during drying can affect the variability and consistency of the nutrient characteristics of the corn ethanol coproducts (Abo-State et al., 2009; Belyea et al., 1998, 2004).

The valuable nutrient compositions of corn ethanol coproducts have motivated researchers to explore the potential incorporation of distillers grains in aquadiet formulations. Many researchers have studied how using DDGS in aquafeed affects the growth performances and biological responses of species such as Nile tilapia, Channel catfish, Yellow perch, and Trout as well as the produced feed physical properties and applied operational conditions for producing the feed. For example, Wu et al. (1994) investigated the effects of three DDGS-based diets containing 36% protein without fish meal as well as 32% protein with and without fishmeal on the growth rate of Nile tilapia, but did not observe any significant difference among the three diets. In a later study, they achieved the highest weight gain and protein efficiency ratio with diets containing 40% protein-35% DDGS and 40% protein-49% DDGS for Tilapia fry, respectively (Wu et al., 1996). In a later study, Schaeffer et al. (2009) suggested that diets with 20% DDGS inclusion can result in a similar biological response to that of the commercial diets for Nile tilapia. Yet another study evaluated the growth response of channel catfish fed a diet containing 30 % DDGS, and no ill effect

was observed (Tidwell et al., 1990). Fish meal replacement with 70% DDGS and 4% lysine supplementation in channel catfish diet was studied and relatively similar results were observed (Lim et al., 2009; Webster, 1991; Zhou et al., 2010). Robinson and Li (2008) studied the effect of replacing fish meal with several plant-based protein for channel catfish and suggested that at least 30% DDGS can be used in the diet when lysine is supplemented. More recently Shaeffer et al. (2011) conducted several feeding trials to explore the growth characteristics (i.e. growth rate, gain weight, feed conversion, and digestibility) of yellow perch fed with diets containing varying amounts of soybean meal and DDGS. They reported that blends with less than 50% DDGS inclusion produced the highest growth performance, but increasing the DDGS portion to 75% lead to a significant decrease in growth response for yellow perch. This study also suggested that a combination of DDGS and SBM can be included in yellow perch diet up to 49.5% with no negative effect. Initial research on the potential inclusion of corn ethanol coproducts (DDGS and DDS) in trout diets was conducted by Philips et al. in 1949 and 1964, respectively. Fowler and Banks (1976) and Hughes (1987) explored the growth characteristics of Rainbow trout fed diets containing corn ethanol coproducts obtained from different production stages. Cheng et al. (2003) explored the effect of combined soybean meal and DDGS inclusion with and without EAAs (methionine) supplementation on trout performance and reported that up to 50% of fishmeal can be successfully replaced by this combination of alternative protein sources while the diet is supplemented with methionine. Later on, Cheng and Hardy (2004) performed a feeding trial to investigate the effect of 7.5%, 15%, and 22.5% DDGS-included diets with and without EAA supplementation, mainly lysine and methionine, on the performance of Rainbow trout. The study suggested that inclusion of 15 %

DDGS without using EAA supplemental and 22.5% DDGS with EAA supplementation may be suitable for trout. Furthermore, in 2008 the USGC reported typical DDGS incorporation rates obtained for various species. Robinson et al. (2009) examined the effect of various corn-based distillers grains on the biological responses (i.e. growth, feed efficiency, and body composition) of channel catfish. When 20 % HP-DDG was used, they did not observe any significant difference in growth characteristics compared to those on the control diet. Very few studies have explored the effect of HP-DDG inclusion on fish growth characteristics for Rainbow trout. Recently, Barnes et al. (2012) conducted a feeding trial for Rainbow trout fed with HP-DDG included feed and examined the effect of HP-DDG along with EAA and phytase supplementations on trout growth and feed conversion. They suggested that up to 20% HP-DDG with amino acid supplementation can be successfully incorporated into trout diets.

As mentioned before, although a well pre-balanced nutritious aquadiet is important to achieve an optimal growth performance, suitable production techniques and well-controlled operational conditions play crucial roles in producing quality aquafeeds (Gokulakrishnana & Bandyopadhyay, 1995). Though not the only manufacturing method, extrusion processing is the most common and economic technology used in the aqua-feed industry. It can improve production productivity and versatility (Kiang, 1998) and can be used for production of floating, sinking and slow sinking aquaculture feed (Opstvedt et al., 2003). An extruder is a bioreactor made of three main parts (i.e. screw, jacketed barrel, and die) in which several unit operations are combined to simultaneously pump, mix, convey, work the raw feed ingredient into a cooked dough, and eventually shape the extruded products through a restricted die using thermal and mechanical energy. The machine's

short processing time helps to retain nutrients in cooked extruded products. This cooking process can also help to detoxify the ingredient blend, inactivate the antinutritional factors and undesired enzymes (Cheftel, 1986; Romarheim et al., 2005), and improve the digestibility, and palatability of the product (Cheftel, 1986; Tran et al., 2008). However, it also may have some detrimental effects in terms of the product's nutritional quality including reduction of protein quality and un-bioavailability of some EAAs due to Maillard reaction and loss of heat-labile vitamins due to exposing to high temperature (Tran et al., 2008). An extruder's performance is greatly influenced by variables such as feed composition, moisture content, barrel temperature, screw speed, mass flow rate, and residence time in the extruder as well as the physics behind the barrel, screw and dies (i.e. configuration and size). As the screw rotates, mixes, and conveys the feed mixture along the barrel, heat is provided either by steam and/or water injection or via an electrical heater in addition to that derived from friction. The interaction of high shear and temperature developing inside the extruder with the present water leads to a complex physiochemical and nutritional alterations in the extruding dough and final products particularly due to gelatinization of starch and denaturation of protein components of the feed blend (Guy, 2001). Hence, applying an appropriately formulated diet composition (Cavalcanti and Behnke, 2005a, 2005b; Faubion et al., 1982; Mathew et al., 1999a, 1999b) along with optimal process conditions (Banerjee and Chakraborty, 1998; Lin et al., 1998; Mathew et al., 1999c; Rolfe et al., 2000) are vitally important in producing physically available and nutritionally viable extruded fish feed (Gokulakrishnana and Bandyopadhyay, 1995; Wood, 1995). Furthermore, both physical and nutritional properties of the extruded aquafeed can vary depending upon the fish species. In terms of the physical properties, water stability, expansion ratio, and

durability index of the extruded feed are very important (Chevanan et al., 2009; Rolfe et al., 2001; Wood, 1995). Generally, the more water stable the extruded feed, the longer the availability of the feed for fish. Since the higher water stability can prevent rapid disintegration of the feed and consequent loss of nutrients when placing it in water. Additionally, the more expanded extrudates exhibit better digestibility due to increased starch gelatinization during the process (Ali, 1998; Case et al., 1992; Gokulakrishnana and Bandyopadhyay, 1995; Rout, 1997; Thomas et al., 1999). Also, the more expanded extrudates float longer which is somehow the main goal in fish feed processing (Vens-Cappell, 1984), though it is not a determining factor as the different cultured ration can be trained to obtain either the floating or sinking food from different depths of the water. On the other hand, the higher the durability index, the more resistant the extrudate against mechanical forces during the storage and transportation (Chevanan et al. 2009; Colonna et al., 1989; Rolfe et al. 2001). In other words, the ultimate quality of the extruded fish feed is a function of feed blend chemical composition and extrusion processing parameters (Ibanoglu et al., 1996; Thomas et al., 1999). In addition, selecting the proper extruder configuration based on the feed ingredients and final properties of the product are key factors in achieving an optimal extrusion process (Tran et al., 2008).

The effects of extrusion processing on the physical properties of DDGS-based aquafeeds have been well documented. Chevanan et al. (2007a) examined the effect of raw blend moisture content, die dimension, and extruder temperature on Nile tilapia diets formulated with 40% DDGS inclusions using a single-screw extruder. They observed that an appropriate combination of blend moisture content, extruder temperature, and die geometry was essential for production of good quality

DDGS-based Nile tilapia feed. In another study conducted by this research group, they investigated the effect of graded levels of DDGS inclusion on extruded Nile tilapia diets produced by a twin-screw extruder and suggested that up to 60% DDGS can be included for production of floating feed (Chevanan et al., 2007b). In a later study, Chevanan et al. (2008) explored the effect of DDGS, moisture content, and screw speed on the physical properties of extrudates in single-screw extrusion. They reported that almost all of the tested physical properties of the final product (such durability, specific gravity, porosity, and color) were significantly influenced by these variables. In a follow-up study, they improved the DDGS-based extrudate quality using the binding properties of whey protein (Chevanan et al., 2009). Later on, they studied the processing behavior of the DDGS-based blends for Nile tilapia and proposed best-fit response surface models for extrusion processing properties including mass flow rate, net torque required, specific mechanical energy consumption, and apparent viscosity (Chevanan et al., 2010). In an attempt to optimize the extrusion processing condition for DDGS-based Nile tilapia diet, Rosentrater and Tulbek (2010) and Fallahi et al. (2011) carried out research to evaluate the effects of conditioner steam, extruder water and screw speed on the final properties of DDGS-based Nile tilapia diets in a pilot-scale twin-screw extruder. Ayadi et al. (2011a, 2011b) conducted two extrusion studies to investigate the extrusion processing effects on DDGS-based yellow perch diets. Using a laboratory scale single-screw extruder at a barrel temperature profile of 40–90–100°C (Feeding zone: 40 °C; conditioning zone: 90 °C; metering zone: 100 °C) and a constant screw speed of 230 rpm, they could produce viable extruded diets for yellow perch with 50% DDGS inclusion (Ayadi et al., 2011a). In a subsequent study using a twin-screw extruder, they achieved higher expansion ratio and

compression strength for the extrudates containing 40% DDGS compared to those of the extrudates produced in their first study (Ayadi et al., 2011b). They also performed another twin-screw extrusion study to produce a DDGS-based diet for Rainbow trout at graded levels of DDGS and concluded that DDGS could be a suitable fish meal replacer in the Rainbow trout diet (Ayadi et al., 2011c).

To our knowledge, no literature result concerning the effects of extrusion cooking process on Rainbow trout diets containing HP-DDG is available. Therefore, the goals of our study are 1) to produce feed for Rainbow trout (*Oncorhynchus mykiss*) using both C- DDGS and HP-DDG as the alternative protein sources and 2) to investigate the physical properties of corn ethanol coproducts protein-based extrudates produced by twin-screw extrusion.

2. Materials and Methods

2.1 Experimental Design and Feed Blend Preparation

Four isocaloric (3.06 kcal/g) experimental diets were formulated with two levels of DDGS (20 and 40%db) and two levels of HP-DDG (20 and 40% db), in combination with calculated amounts of the other required dietary ingredients including corn gluten meal, wheat flour, vitamin mix, minerals, and essential amino acids, to contain a net protein content of ~40% db. Table 1 lists the percent ingredient composition (dry basis percent) for the experimental diets for rainbow trout and the control feed. A fishmeal based diet (100% fishmeal as the protein source) was used as the control feed. DDGS and HP-DDG were provided by Dakota Ethanol (Wentworth, SD), and were ground to a fine particle size of approximately 100 µm with a laboratory-scale grinder (Model S500 disc mill, Gen Mills, Clifton, NJ). Corn gluten meal from Consumers Supply Distributing Company

(Sioux City, IA); wheat flour from Bob's Red Mill Natural Foods, Inc. (Milwaukie, OR); CMC from USB Corporation (Cleveland, OH); vitamin premix and minerals were from Lortscher Agri Service, Inc. (Bern, KS). The ingredients were mixed with a laboratory-scale mixer (Model 600, Hobart Corporation, Troy, OH) for 3 min; then, the vitamin premix was added to the rest of the ingredients, and the blend was mixed with a twin shell dry blender (The Patterson-Kelly Co. Inc., East Stroudsburg, PA) at 60 rpm for 10 min to produce homogenous blends. The resulting blends were then stored at ambient temperature overnight.

2.2 Extrusion Processing

The experimental extrusion cooking process was performed using a semi industrial, co-rotating, self-wiping, and fully intermeshing twin-screw extruder (Wenger TX-52, Sabetha, KS) which had a 30 hp motor and throughput of 50 - 250 kg / h. Using a dry feed hopper, the ingredient blends were transferred and conveyed into a continuous preconditioner where the ingredients were mixed and the desired moisture content and temperature were adjusted via steam injection at a rate of 0.11-0.16 kg/min. Then, the blends were transferred into the extruder at a feeder rate of 20 kg/h. The screws' diameter and L/D ratio of the extruder barrel were 52 mm and 25.5:1, respectively. The screws used in this experiment had 25 individual sections, and the configuration from the feeding to the die section, was composed of: four conveying screws, three shear locks, one conveying screw, one conveying screw backward, three conveying screws, one conveying screw backwards, four conveying screws, one shear lock, one interrupted flight conveying screw, one conveying screw, one interrupted flight conveying screw, one conveying screw, one interrupted flight conveying screw, one shear lock, and finally a screw with a cone-shaped end point. Moreover, the barrel was

composed of eight temperature zones which were set at 25 to 90 °C. The temperature profile of the barrel varied, depending on the actual temperature of each zone and the extrudates' properties. The amount of water added to the extruder was maintained at 0.11 to 0.19 kg/min. It had two die nozzles each of which with circular opening of 3 mm. Exiting the die section, the extrudates were cut into desired lengths, using a three blade cutter.

2.3 Measurement of Extrusion Processing Parameters

2.3.1 Temperature (T):

Temperatures of the raw blends inside the hopper, at the conditioner exit and die zone were all monitored by a portable infrared thermometer (Model 42540, Extech Instruments Corporation, Waltham, MA).

2.4 Measurement of Extrudate Physical Properties

The extruded diets were cooled for 72 h at ambient temperature ($24 \pm 1^\circ\text{C}$) and dried in an oven (Model TAH-500, Grieve Corporation, Round Lake, IL) for 24 h at 45°C . Then, the dried extrudates were subjected to extensive physical property analyses, including moisture content (MC), water activity (a_w), thermal conductivity (K), thermal resistivity (R), thermal diffusivity (α), expansion ratio (ER), unit density (UD), bulk density (BD), water absorption and water solubility indices (WAI, WSI), pellet durability index (PDI), and color.

2.4.1 Moisture content (MC):

MC of the extrudates was determined according to AACC method 44-19 (2000), using a laboratory-scale oven (Fischer Scientific) at 135°C for 2h.

2.4.2 Water activity (a_w):

Water activity of the extrudates was measured with a water activity meter (a_w Sprint TH-500, Novasina, Pfäffikon, Switzerland). The system was calibrated according to the procedure specified by the manufacturer.

2.4.3 Thermal properties:

Thermal conductivity (k), thermal diffusivity (α), and thermal resistivity (R) were determined using a thermal properties analyzer (KD2, Decagon Devices, Inc., Pullman, WA).

2.4.4 Expansion ratio (ER):

The diametral expansion of the extrudates was determined as the ratio of extrudate diameter (mm) to the diameter (mm) of the die nozzle, using a digital caliper (Digimatic Series No. 293, Mitutoyo Co., Tokyo, Japan.), following Conway and Anderson (1973) and van Zuilichem et al. (1975).

2.4.5 Unit density (UD):

Assuming cylindrical shapes for the extrudates, UD was determined as the ratio of the mass (g) to volume (cm^3) for ten randomly chosen extrudates, following Jamin and Flores (1998) and Rosentrater et al. (2005). The mass of each of the extrudates was measured with an analytical balance (Adventurer. AR 1140, Ohaus Corp. Pine Brook, NJ) and the diameter of each was measured with a digital caliper (Digimatic Series No. 293, Mitutoyo Co., Tokyo, Japan).

2.4.6 Bulk density (BD):

Using a standard bushel tester (Seedburo Equipment Company, Chicago, IL), BD (g/cm^3) was measured as the ratio of the mass of the extrudates (g) occupying a given bulk volume, to the volume of the bulk (0.5 L) (USDA, 1999).

2.4.7 Water absorption and water solubility indices (WAI, WSI):

Using a laboratory-scale grinder (Chemical Rubber Co, CRC, Germany) extrudate samples were ground to a particle size of approximately 150 μm , then 2.5 g of the finely ground sample were placed in a 50 mL centrifuge tube, and 30 mL distilled water at 30 $^{\circ}\text{C}$ was added to the tube. After intermittently stirring for 30 min, the suspension was centrifuged at 3000 X g for 15 min using a laboratory-scale centrifuge (accuSpinTM 400, Thermo Electron Corporation). Thereafter, the supernatant phase was transferred into aluminum dishes and placed in a laboratory oven (Fisher Scientific) at 135 $^{\circ}\text{C}$ for 2h. WAI was calculated as the mass ratio of the remaining gel in the centrifuge tube to the original mass of the sample, equation (1):

$$WAI(-) = [\text{Mass of gel}]/[\text{Mass of sample}] \quad (1)$$

where masses were determined in g.

Subsequently, WSI (%) was calculated as the mass ratio of the extracted dry solid to the original sample mass, following Anderson et al. (1969) and Jones et al. (2000).

2.4.8 Pellet durability index (PDI):

PDI was determined following ASAE standard method S269.4 (ASAE, 1996); 200 g of the extruded sample was tumbled inside a PDI tester (model PDT -110, Seedburo Equipment Co., Chicago, IL) for 10 min, and then sieved manually via a No. 6 screen. PDI was calculated with equation (2), where M_a and M_b are the mass (g) of the extrudates after tumbling and before tumbling, respectively:

$$PDI(\%) = \frac{M_a}{M_b} \times 100 \quad (2)$$

2.4.9 Color determination (L^* , a^* , and b^*):

Brightness/darkness (L^*), redness/greenness (a^*), and yellowness/blueness (b^*) were measured using a spectrophotometer (Lab Scan XE, Hunter Lab, Reston, VA).

3. Statistical Analysis

All measurements were made in triplicate, except for UD and ER, where ten measurements were taken. All collected data were analyzed with Microsoft Excel v.2010 and SAS v.9.0 software (SAS Institute, Cary, NC) using a Type I error rate (α) of 0.05, by analysis of variance (ANOVA) to find if there were significant differences among control diet and experimental diets. Then post-hoc LSD tests were used to determine where the specific differences occurred ($p < 0.05$).

4. Results and Discussion

4.1 Extrusion Processing Parameters

4.1.1 Dough temperature during extrusion processing (T):

Temperatures at three points of the process were monitored. As shown in Table 3, substitution of DDGS and HP-DDG significantly influenced the measured processing temperatures. Inclusion of 20% DDGS and 20 % HP-DDG led to a 3.1 % and 7.2 % increase in processing temperature at conditioner section (T_C), respectively. Further addition of DDGS and HP-DDG up to 40%, resulted in 4.6% and 5% increase in T_C , respectively. While this increase caused a 5.2% and 2.5 % increase in T_d compared to that of the control diet. Increasing DDGS and HP-DDG from 20 % to 40%, no considerable changes were observed for T_C and T_d of the extrudates. Temperature variation inside the barrel can affect the performance of extrusion process as well as the final extrudates qualities. Proportional increasing rate of temperature along the extruder length can be ascribed to both heat addition as well as frictional heating. Also, the temperature development inside the barrel can vary depending upon the thermal properties of the dough (i.e. thermal conductivity and thermal diffusivity), degree of mixing, and velocity of dough flow (Chevanan et al., 2007a).

4.2 Measurement of extrudate physical qualities

All the measured physical properties of the final products including moisture content, water activity, thermal properties, expansion ratio, unit density, bulk density, water absorption and water solubility indices, pellet durability index, and color are provided in table 4.

4.2.1 Moisture content (MC):

Water is one of the most important components of a biological substance playing crucial roles in forming the various properties such as mechanical, textural, and biological properties (Blahovec, 2007). It is also one of the main governing factors in food and feed processes like extrusion cooking

process which affects the processing behavior and the final products qualities. In general, water or moisture content of a substance can exist in the form of bound, unbound, and free water (Mohsenin, 1986). In fact, the effect of free water content on the physical and biological properties of either raw and un-processed materials or processed and final products of a process is predominant. As per definition, free moisture content within a substance is the amount of moisture higher than the equilibrium water content which can be removed by drying under a certain relative humidity. Free water content of a substance can act as a plasticizer and impact the mechanical properties of a material such as viscoelasticity (Blahovec, 2007; Rosentrater et al., 2009). In extrusion processing, the synergistic effect of free water content of the ingredient and developed high temperature and high shear forces inside the extruder greatly can change the dough characteristics and extrudates physical properties like cohesiveness, water holding capacity, water stability, and storage stability (Chevanan et al., 2010a; Rolfe et al., 2001). On the other hand, biochemical reactions among the water and chemical constituents of the blend in which starch, protein, and lipid contents play the most important roles, are profoundly influenced by the interaction effects of thermomechanical phenomena like heat transfer, shearing forces, and high pressure developed inside the barrel of the extruder during the process. The most effective reactions occurred during the process are starch gelatinization and protein denaturation which are drastically influenced by the amount and the source of these components (Mercier et al., 1989; Miller, 1985; Riaz, 2000). Due to the combination effects of these biochemical and thermomechanical alterations paired with a drastic and sudden pressure drop among the die entrance, die exit, and atmosphere, free water content of the molten dough evaporates and the latent heat of vaporization changes the internal structure of the exiting

extrudate and eventual properties of the products (Friesen et al., 1992). Additionally, water content of a material is a function of the water activity of the material and the humidity of the surrounding air (Blahovec, 2007). Furthermore, the amount of free water content of a biological material plays a key role in the microbial growth and thus spoilage of the material (Abramovic and Mojca, 2008).

As depicted in table 4, inclusion of 20% DDGS and 20% HP-DDG resulted in 7% and 36 % decrease in MC of the extrudates compared to that of the control diet, respectively. Further increasing of the DDGS and HP-DDG to 40% dropped the MC of the extrudates by 31% and 45%, respectively. No significant influence on MC of the products was observed between the diets containing 40% DDGS and 20% HP-DDG.

4.2.2 Water activity (a_w):

Water activity is a crucial consideration for food and bio-based materials safety which serves for the explanation of the material's potential for microbial and fungal deterioration, and it is defined as the available water for microorganism use which affects the food safety and storage shelf life of the products (Abramovic and Mojca, 2008). The water availability within a material can change with temperature variation as it can affect the water binding (Higl et al., 2007); thus, a_w of a biological material can impact the enzyme activity and at some point, the nutritional value of the food product such as vitamin availability.

Using 40% DDGS, 20% and 40% HP-DDG in the diet formula reduced the a_w of the extrudates by more than 50% compared to that of the control diet. Inclusion of 20% DDGS reduced the a_w of the extrudates only by 10%. The average water activities of all the extrudates lay below 0.25, except that of diet 2 which was 0.43.

4.2.3 Thermal properties:

Knowledge about the thermal properties of raw ingredients and final products of a thermal process can provide valuable information about the heat transfer through the system and the energy requirements needed at various stages of the thermal process. Three thermal properties of the extruded diets including thermal conductivity (k), thermal diffusivity (α), and thermal resistivity (R) were measured and the data are reported in table 4. Thermal conductivity of a material is a function of temperature gradient (Willix et al., 1998) and can be ascribed by the material ability to transfer the heat through itself without any motion. Sablani et al. (2002) suggested that k is a function of the moisture content, temperature, and apparent density for the baked products. It is believed that physiochemical reactions among the blend constituents, occurring above a certain temperature range, during a thermal processing, depending on the material nature can reduce k value of the heat-processed proteinaceous and starchy materials due to the protein denaturation and starch gelatinization and thus creation of highly cross-linked macromolecule structures (Fessas and Schiraldi, 2000; Heldman, 2003). Addition of more hydrophobic ingredients in the raw blend can decrease the k value of the heat processed products such as extrudates. This explains why thermal properties of the extrudates are highly related to the initial components of the blend, process operational conditions, and internal structure of the extrudates (Alavi et al., 1999). In our extrusion study, neither inclusion of DDGS nor HP-DDG changed the k value of the extrudates compared to that of the control diet.

On the other hand, R is an indicator of a material ability to store the heat (Kawasaki and Kawai, 2006) which can also vary as a function of material thickness and temperature gradient (Arambula-

Villa et al., 2007). Addition of 20 % and 40 % DDGS decreased R of the extrudates by 5.0 % and 3.6%, respectively. While addition of 20% HP-DDG resulted in a 3.5 % increase in the R, further increasing of HP-DDG to 40% did not have any significant impact on the heat storage capacity of the extrudates. Regarding the α values of the extruded diets, our observations indicated that inclusion of 20% DDGS decreased the α by nearly 6.3%, while further addition of DDGS to 40% and inclusion of HP-DDG at both levels of 20% and 40% did not influence the α value, considerably compared to that of the control diet. The lowest α value of 0.15 was related to the less expanded extrudates containing 20% DDGS with ER of 1.043. This was in agreement with our observations in another study conducted for soybean meal-based Yellow perch extruded diets (Fallahi et al., 2012). These results can be ascribed to the higher porosity of the more expanded extrudates and the higher thermal diffusivity of the trapped air in the pores compared to those of the protein and starch (Mariam et al., 2008). Ayadi et al. (2011a) suggested that the low k value and α value of the raw blends may affect the extrusion processing adversely due to a longer time required for heat transfer during the cooking process. On the other hand, the substances with low heat conductivity and diffusivity may be more heat-stable and thus less affected by external thermal sources. They also believed that, the low values of k, R, and α can impact the required time for cooling and/or drying of the extrudates. In general, knowledge of the thermal properties of the ingredients can be helpful to control the extrusion operational conditions and to manipulate the product physical properties such as expansion ratio and density.

4.2.4 Expansion ratio (ER):

One of the most critical factors indicating the physical quality of the aquafeed is the expansion ratio influencing the floatability of the feed. As depicted in table 4, inclusion of 20% and 40% DDGS resulted in a significant decrease in extrudate ER by nearly 11.6% and 4.2%, compared to that of the control diet, respectively. Also, it is clear that inclusion of HP-DDG at both levels of 20% and 40% led to the production of more expanded and floatable extrudates compared to those extrudates including DDGS; however, no significant change in ER values of HP-DDG included extrudates were observed compared to that of the control diet. The higher ER of the extrudates from Diet 3 and 4 could be due to the lower crude fiber content of the HP-DDG compared to that of DDGS and consequently less water desorption by the HP-DDG included extrudates. All the produced extrudates were floated for more than 25 min (Pic 1). Generally, there is a positive relation between an extrudate's ER and floating property (Rolfe et al., 2001; Rosentrater et al., 2009). The more expanded extrudates imply the more gelatinization of the starch component of the extruding blend which can result in a crispier and easier to digest extrudates (Rout, 1997). It is expected that, as the starch content is decreased, the obtained ER during extrusion is reduced as well (Chevanan et al., 2009). Obtaining a desired ER is one of the challenging issues when corn ethanol coproducts are used as the fishmeal replacers (Chevanan et al., 2009). In fact, numerous factors may influence the extent of expansion during the extrusion processing. For instance, the magnitude of the pressure gradient generated at the die exit during the evaporation can impact the ER of the extruding melted dough; this pressure gradient causes water phase transition and indeed is a driving force for expansion phenomenon and formation of puffy texture in the extrudates (Alves et al., 1999; Lam and Flores, 2003; Moore, 1990); however, its magnitude is proportional to the entire feed

constituents, dough moisture content, and die configuration (Bouzaza et al., 1996; Chevanan et al., 2007a; Tumuluru and Sokhansanj, 2008). Additionally, feeding rate, rheological behavior of the extruding dough, residence time (Fan et al., 1994; Chevanan et al., 2007a; Mitchell et al., 1994), and extrudates mass flow rate (Chevanan et al., 2007a; Tumuluru and Sokhansanj, 2008) can affect the extent of developed pressure gradient at the die exit. On the other hand, dynamic flow of the dough inside the die is drastically influenced by the temperature and the present water content. The high temperature and lubricant effect of water increase the molecules mobility and thus the melted dough exhibits pseudoplastic behavior leading to a substantial decrease in the apparent viscosity of the extruding dough which affects the extent of pressure release and ultimately reduces ER of the finished product (Chevanan et al., 2007a; Mjoun and Rosentrater, 2011; Rosentrater et al., 2005). While the higher the starch content results in a more expanded extrudates due to starch gelatinization and elastic characteristic of the dough inside the barrel and consequent pressure rise at the die exit (Ibanoglu et al., 1996; Kokini et al., 1992; Lin et al., 2000; Nielsen, 1976; Sokhey et al., 1994); the more incorporation of protein in the blend ingredients results in production of extrudates with more porous texture mainly because of the protein denaturation and plastic characteristic of the melted dough inside the extrusion barrel (Chevanan et al., 2007a; Sandra and Jose 1993; Singh et al., 1991). The ability of the protein component of the blend in distribution of water inside its macromolecular structure can also affect the ER of the extrudates (Deshpande and Poshadri, 2011). Also, applying extrusion conditions with the higher barrel temperature causes more excretion of super-heated steam and subsequently more expanded products will be produced (Kim et al., 1989).

4.2.5 Unit density (UD):

UD is the other important physical properties of the extruded aquafeed which is directly related to the ER of the extrudates and thus impacts the floatability of the feed (Chevanan et al., 2007a, 2009; Colonna et al., 1989). Therefore, UD of the extruded products can be influenced by the factors impacting ER. Several extrusion studies have reported that there is a negative correlation between the barrel temperature during the extrusion process and UD of the extruded product (Bhattacharya and Hanna, 1986; Harper et al., 1981) which implies the pseudoplastic behavior of the melted dough, decrease in apparent viscosity, less pressure gradient at the die exit, subsequent increase in expansion, and thus reduction in UD of the extrudates. Their observations were in agreement with what Chevanan et al. (2007a) reported regarding the inverse effect of barrel temperature on UD of the DDGS-based extrudates. Comparison of the UD of the extrudates from the four experimental diets to that of the control diet, exhibited that DDGS inclusion at level of 20% had the greatest effect on UD and led to a significant increase in UD value by 21.20 % where further increasing of DDGS to 40% and inclusion of HP-DDG at both levels of 20% and 40% slightly increased the UD values of the extrudates which were not statistically considerable (Table 4). As shown, the minimum UD value of 731.70 (kg/m³) was attained for the control diet while the highest value of 886.65 (kg/m³) was related to the extrudates from diet 1 (Table 4). Overall, UD values were less than that of the water which confirms the buoyancy of the extrudates.

4.2.6 Bulk density (BD):

As the other key physical properties of biological products, BD always has been one of the major factors to consider. It is very important for assessing the appropriate packaging, the required storage

space for warehousing and transportation of the products as it indicates the mass content of a given volume of product (Guy, 2001; Rosentrater, 2006). From an economic point of view, the higher the bulk density, the better, because it reduces the packaging, storage and transportation costs for a certain mass of product. Similar to UD and ER, BD of the extrudates is influenced by the effect of extrusion processing conditions on thermodynamic characteristics of the ingredient mixture. For example, screw speed, barrel temperature, die dimension, and feed moisture content are some of these parameters that significantly influence the extruding dough temperature, gradient pressure at die section, dough density, dough viscosity, and consequently extrudates UD and BD values (Tumuluru and Sokhansanj, 2008).

As shown in Table 4, the highest BD of 666.91 (kg/m³) was observed for the extrudates with 20% DDGS inclusion (Diet 1) with a 16.6 % increase compared to that of the control diet. While further inclusion of DDGS to 40% led to only 5% increase in BD value of the extrudates. There was no significant difference among the BD values of products with 20% and 40% HP-DDG and that of the control diet. These observations for the decreasing and increasing values of ER and UD of the extrudates with 20% DDGS inclusion confirm the inverse and direct relation of BD to ER and UD, respectively. As shown in the results, the most floatable extrudates had the lowest BD and UD values. On the other hand the higher the BD the better the products; therefore, making a balance between the desired ER and BD values of the products is the other major factors in aquafeed industry that need to be considered (Mjoun & Rosentrater, 2011).

4.2.7 Water absorption and water solubility indices (WAI, WSI):

Table 4 shows that the water absorption index values were more for the extrudates made from diets containing DDGS than that of the extrudates produced from HP-DDG based diets. It could be due to higher starch content of the DDGS. Incorporation of 20% DDGS yielded an 11% increase in WAI and further increasing of DDGS to 40% raised the WAI by nearly 21%, while using 20 % and 40 % HP-DDG reduced the WAI by 30 % and 26 % compared to that of the control diet, respectively. The maximum WAI of 4.21 was observed for the extrudate made from diet 2. No significant difference was observed between the WAI values of the products produced from diet 3 and 4. Looking at data presented in Table 4, the extrudates with higher BD also had relatively higher WAI. It was in contrast with our observations in a previous twin-screw extrusion study performed for soy-protein based Yellow perch diet (Fallahi et al., 2012). Also, there was no relation between the ER and WAI of the extrudates. While, in another study conducted by Adeparusi and Famurewa (2011), a direct relation between the ER and WAI of the extrudates was reported. They suggested that the more expanded extrudates can absorb more water. Theoretically, the ability of the extrudates in absorption of water can be attributed to the un-damaged portion of starch content of the feed blend which was not influenced by the extrusion processing (Chevanan et al., 2007a ; Govindasamy, 1996; Mason and Hosney, 1986). In other words, the greater the WAI of the extrudates implies lesser change in native structure of the starch components of the feed blend and consequently, lesser depolymerization of amylose and amylopectin molecules during extrusion processing. However, many researchers suggested that any structural modification such as starch gelatinization and protein denaturation in the macromolecule components of the blend can cause significant change in WAI of the extrudates (Badrie and Mellowes, 1991; Chevanan et al., 2007a; Rosentrater et al.,

2009). Additionally, intensity of heat treatment causes those water binding sites on the side chain of the protein molecules which were blocked by the lipid to be more available to the water and thus the water absorption increased (Sathe et al., 1982).

In terms of the WSI, we observed that inclusion of DDGS at both levels of 20% and 40% slightly increased the WSI compared to that of the control diet while incorporation of 20 % HP-DDG and 40 % HP-DDG significantly increased the WSI by approximately 13 % and 9 %, respectively.

Extrudates produced from diet 3 and 4 with HP-DDG inclusion exhibited to have higher WSI values compared to those of the diet 1 and 2 produced from C-DDG based feed blends. As it was expected (Anderson et al., 1982), an inverse relation between the WSI and WAI values of the extrudates was observed. Since in contrast to WAI, the magnitude of the WSI of the extrudates reflects the percentage of the portions of starch and/or protein molecules of the feed blend which were drastically degraded during the extrusion processing (Colonna Mercier, 1983; Govindasamy, 1996). The extrudates with the highest WSI of 16.70 (%) had the lowest WAI of 2.45 (-) which were produced from diet 3 with 20% HP-DDG. In fact, the nature of the protein and starch components of the feed blend paired with the combination effects of extrusion processing parameters such as barrel temperature, developed shearing forces, pressure, and even the physical configurations of the screw, barrel chamber, and the die section influence the extent of depolymerization and water plasticization of these macromolecules and thus alter the WSI and WAI values of the extrudates (Anderson et al., 1982; Menegassi et al., 2011).

4.2.8 Pellet durability index (PDI):

Durability index of the extruded feed is the other physical properties predicting the mechanical strength of the extrudates against the abrasive forces specifically during the transportation, storage and feeding. Indeed, engineering the PDI greatly helps to reduce the economic losses that may occur due to nutrient loss or pond pollution as a result of using low quality extrudates with mechanically weak structure. Physiochemical transformation of starch component of the feed blend in response to the variation of extrusion processing condition significantly impacts the cohesiveness and durability of the extruded blends against the external pressures (Chevanan et al., 2007; Colonna et al., 1989; Rosentrater et al., 2009). As depicted in Table 4, all measured PDIs lay above 99%, irrespective of protein source types and their inclusion levels. The diet with 20 % DDGS had the lowest PDI of 99.33% and the diet with 40 % HP-DDG reached the highest PDI of 99.64 %; however, there were no statistical differences between the PDI values of the experimental diets compared to that of the control diet. Similar results were attained by Rosentrater and Tulbek (2010), Ayadi et al. (2011a, 2011b, and 2011c), and Fallahi et al. (2011).

4.2.9 Color (L^* , a^* , and b^*):

Color is another physical property of the food products which can be used for indirectly verification of safety (Friedman, 1996) and nutritional quality (Berset, 1989; Dahl and Villota, 1991; Rosentrater et al., 2005) of the food. Color of biological products is drastically influenced during the thermal processing mainly due to the non-enzymatic browning reaction (Maillard) which leads to protein deterioration. In extrusion processing, high temperature and semi-dry condition favors the browning reaction which results in amino acid destruction and unavailability of some of essential amino acids such as lysine and tryptophan (Hurrell et al., 1979). The required reduced sugar for this

process is provided by hydrolysis and dextrinization of starch component of the feed blend induced by mechanical shearing (Shagun and Harper, 1980). Other factors such as screw configuration and size of the die opening which impact the extent of shear forces can cause significant physio-chemical alteration in protein, formation of deleterious compounds, and change the extrudate color (Friedman, 1996; Mega and Cohen, 1978). As the amino acid lysine produces the most color when reacting with sugars, change of extrudate color can be attributed to the formation of unusable blocked lysine (Chevanan et al. 2007a) due to crosslinking the protein (Feeney, 1980; O'Brien and Morrissey, 1989). Guerra-Hernandez et al. (1999) claimed that the intensity of the browning reaction can be quantified by measuring the color change of the heat treated products compare to raw materials.

The effects of each diet on color of the extrudates are provided in Table 4. In general, inclusion of 20 % DDGS resulted in 7.6 %, 12.2 %, and 12.9 % decrease in lightness (L^*), redness (a^*), and yellowness (b^*) of the extrudates compared to those of the control diet, respectively. Further incorporation of DDGS to 40 % led to a 2.8 % and 5.8 % increase in L^* and b^* values, respectively, while it did not show any effect on a^* of the produced extrudates compared with those of the control diets. Also, inclusion of 20 % and 40 % HP-DDG yielded an increase of 3.5 %, 1.8 %, 5.9 %, and 2.9 % in lightness and yellowness but a decrease of 4.5 % and 7.3 % in redness of the extrudates, compared to those of the control diet, respectively.

5. Conclusions and future study

The results from this twin-screw extrusion study revealed that, replacing fishmeal with HP-DDG at both levels of 20 and 40 % led to production of extrudates with higher ER and WSI values but

lower UD, BD, and WAI values compared to those of the diets produced with 20 % or 40 % DDGS incorporation. Using 20 % DDGS resulted in production of the extrudates with minimum ER, L^* , a^* , and b^* but maximum UD and BD values compared to those of the fish meal-based diet. Extrudates with 20 % HP-DDG inclusion appeared to have the highest darkness value (L^*). Increasing inclusion levels of HP-DDG did not show any effect on UD or ER of the products. Clearly, the inclusion of HP-DDG at both levels of 20% and 40% led to the production of more expanded and floatable extrudates compared to those extrudates contained DDGS. All the extrudates of four experimental diets exhibited to have excellent durability index indicating that DDGS and HP-DDG inclusions do not have ill effects on the extruded diet resistance against the abrasive forces. Water activity of all the extrudates lay below 0.45. Low a_w of the produced extrudates guaranteed a stable product with long storage stabilities. Overall, DDGS and HP-DDG up to 40 % could be used to produce quality extrudates with acceptable physical properties for rainbow trout. This study was a positive step toward the utilization of HP-DDG based feed in aquaculture industry.

Future research should concentrate on examining the effects of both extrusion processing and higher levels of HP-DDG on rainbow trout feeds, as well as nutritional efficacy by conducting feeding trials.

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Table 1: Ingredient components (g/100g) of the feed blends.

Components (%db)	Control	Diet 1	Diet 2	Diet 3	Diet 4
Dry weight of ingredients (g/100g)					
HP-DDG ^a	0.00	0.00	0.00	11.08	22.21
DDGS ^b	0.00	11.00	20.94	0.00	0.00
Fish meal ^c	45.90	45.00	33.71	45.57	34.10
Corn gluten meal ^d	28.07	24.30	24.19	22.30	22.25
Whole wheat flour ^e	22.47	14.45	12.10	12.27	12.25
CMC ^f	0.76	0.57	0.84	0.46	0.88
Vitamin premix ^g	0.63	0.62	0.62	0.62	0.62
Mineral mix ^h	0.00	0.12	0.12	0.12	0.12
Oils	$\frac{3}{4}$	$\frac{3}{4}$	$\frac{3}{4}$	$\frac{3}{4}$	$\frac{3}{4}$
Supplements ⁱ (total from below)	2.17	3.94	7.47	7.58	7.56
Stay-C	0.63	0.62	0.62	0.62	0.62
Choline	0.00	0.00	0.00	0.00	0.00
Phytase	0.00	0.05	0.05	0.05	0.05
DVAqua	0.16	0.16	0.15	0.16	0.16
Arginine	0.00	0.00	0.00	0.00	0.00
Lysine	0.00	0.62	0.62	0.62	0.62
Isoleucine	0.00	0.37	0.37	0.37	0.37
Histidine	0.00	0.12	0.12	0.12	0.12
Glycine	0.00	0.00	0.00	0.00	0.00
Methionine	0.00	0.62	0.62	0.62	0.62
Taurine	0.00	0.00	0.00	0.00	0.00
Sodium chloride	0.63	0.62	1.11	1.12	1.12
Potassium chloride	0.76	0.75	0.86	0.87	0.87
Magnesium oxide	0.00	0.00	0.12	0.12	0.12
Calcium phosphate	0.00	0.00	2.84	2.87	2.87
Total	100.00	100.01	100.00	100.00	100.00

^a HP-DDG, Dakota Ethanol (Wentworth, SD)^b DDGS, Dakota Ethanol (Wentworth, SD)^c Menhaden fish meal, Omega Protein Inc (Houston, TX).^d Corn gluten meal, Consumers Supply Distributing Company (Sioux City, IA)^e Whole wheat flour, Bob's Red Mill Natural Foods, Inc. (Milwaukie, OR)^f Carboxyl methyl cellulose (CMC), USB Corporation (Cleveland, OH)^g Vitamin premix, Lortscher Agri Service, Inc. (Bern, KS)^h Mineral, Lortscher Agri Service, Inc. (Bern, KS)

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Table 2. Proximate compositions of the diets (%db)

Composition (%db)	Diet				
	Control	Diet 1	Diet 2	Diet 3	Diet 4
Crude Protein	39.96	41.89	38.05	42.03	39.79
Crude Fat	16	16.25	16.28	16.18	16.24
Crude Fiber	0.36	0.36	0.36	0.36	0.36
Ash	2.03	2.03	2.63	2.63	2.63

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Table 3. Treatment effects on extrusion processing parameters.

Parameter	Treatment				
	Control	Diet1	Diet 2	Diet 3	Diet 4
Processing temperature (°C)					
Feeder zone	25.44 ^c (0.20)	26.32 ^b (0.29)	26.72 ^a (0.05)	26.75 ^a (0.18)	26.33 ^b (0.46)
Conditioner zone	30.76 ^b (0.53)	31.72 ^a (0.74)	32.18 ^a (0.83)	32.96 ^a (0.65)	32.34 ^a (1.13)
Die zone	46.02 ^b (1.03)	47.66 ^a (0.43)	48.40 ^a (0.25)	48.28 ^a (0.28)	47.18 ^a (1.01)

Mean values among treatments followed by similar letters for a given dependent variable are not significantly different at $P < 0.05$. Values in parentheses are standard deviation.

Table 4. Main effects on extrudate physical properties.

Property	Treatment				
	Control	Diet1	Diet2	Diet 3	Diet 4
MC (%)	8.32 ^a (0.12)	7.74 ^b (0.49)	5.76 ^c (0.34)	5.30 ^c (0.79)	4.60 ^d (0.32)
a _w (-)	0.48 ^a (0.01)	0.43 ^b (0.01)	0.24 ^c (0.01)	0.23 ^d (0.01)	0.21 ^e (0.02)
k (W/m·C)	0.056 ^a (0.01)	0.056 ^a (0.01)	0.056 ^a (0.01)	0.052 ^a (0.00)	0.055 ^a (0.00)
R (m·C /W)	18.47 ^{ab} (0.89)	17.50 ^b (0.85)	17.80 ^b (0.39)	19.11 ^a (1.14)	18.42 ^{ab} (0.66)
α (mm ² /s)	0.16 ^{ab} (0.01)	0.15 ^c (0.01)	0.16 ^b (0.00)	0.17 ^a (0.01)	0.16 ^{ab} (0.01)
ER (-)	1.18 ^a (0.08)	1.043 ^c (0.05)	1.13 ^b (0.02)	1.16 ^{ab} (0.07)	1.16 ^{ab} (0.04)
UD (kg/m ³)	731.70 ^b (100.11)	886.65 ^a (89.82)	749.58 ^b (36.62)	755.72 ^b (90.45)	750.92 ^b (75.35)
BD (kg/m ³)	572.15 ^c (1.29)	666.91 ^a (2.05)	601.13 ^b (0.29)	575.89 ^c (5.89)	583.98 ^c (22.78)
WAI (-)	3.48 ^c (0.06)	3.87 ^b (0.10)	4.21 ^a (0.18)	2.45 ^d (0.10)	2.58 ^d (0.27)
WSI (%)	14.81 ^b (0.24)	15.17 ^{ab} (0.39)	15.05 ^{ab} (0.52)	16.70 ^a (2.74)	16.16 ^{ab} (1.80)
PDI (%)	99.42 ^{ab} (0.05)	99.33 ^b (0.45)	99.54 ^{ab} (0.03)	99.59 ^a (0.18)	99.64 ^a (0.07)
L* (-)	22.36 ^c (0.21)	20.66 ^d (0.20)	22.99 ^{ab} (0.14)	23.14 ^a (0.44)	22.78 ^b (0.39)
a* (-)	5.50 ^a (0.10)	4.83 ^c (0.10)	5.50 ^a (0.09)	5.25 ^b (0.12)	5.10 ^b (0.32)
b* (-)	8.99 ^c (0.21)	7.83 ^d (0.09)	9.52 ^a (0.09)	9.52 ^a (0.14)	9.25 ^b (0.26)

MC is moisture content; a_w is water activity; K is thermal conductivity; R is thermal resistivity; α is thermal diffusivity; ER is expansion ratio; UD is unit density; BD is bulk density; WAI is water absorption index; WSI is water solubility index; PDI is pellet durability index; L* is brightness /darkness; a* is redness/greenness; b* is yellowness/blueness; Parentheses indicate ± 1 standard deviation; Means followed by similar letters for a given dependent variable are not significantly different at P<0.05 among treatments.

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Picture 1. All extrudates from diet 1 (20% DDGS), diet 2 (40% DDGS), diet 3 (20% HP-DDG), and diet 4 (40% HP-DDG) floated for more than 25 min.